

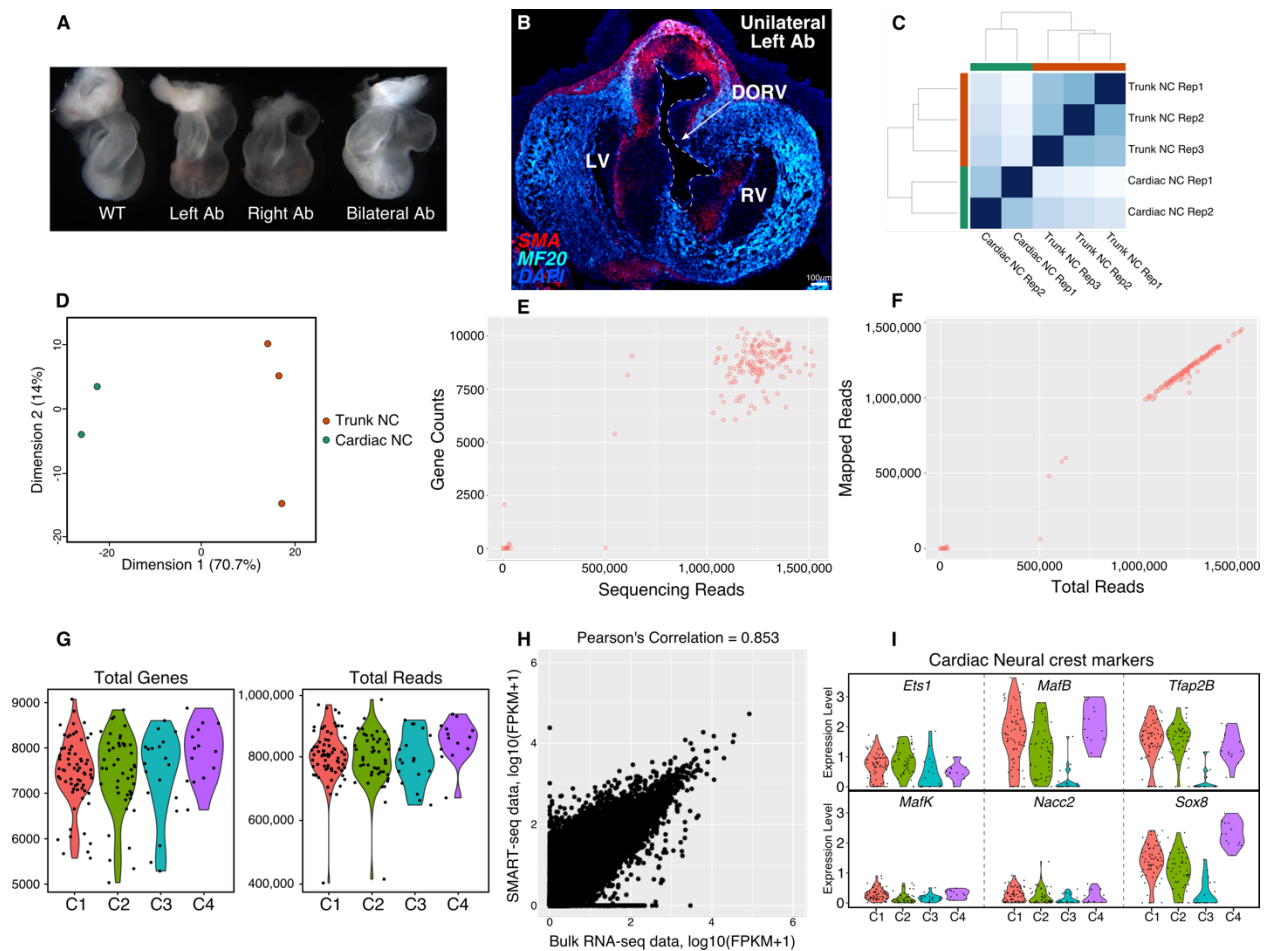
Developmental Cell, Volume 53

Supplemental Information

**Reprogramming Axial Level Identity
to Rescue Neural-Crest-Related
Congenital Heart Defects**

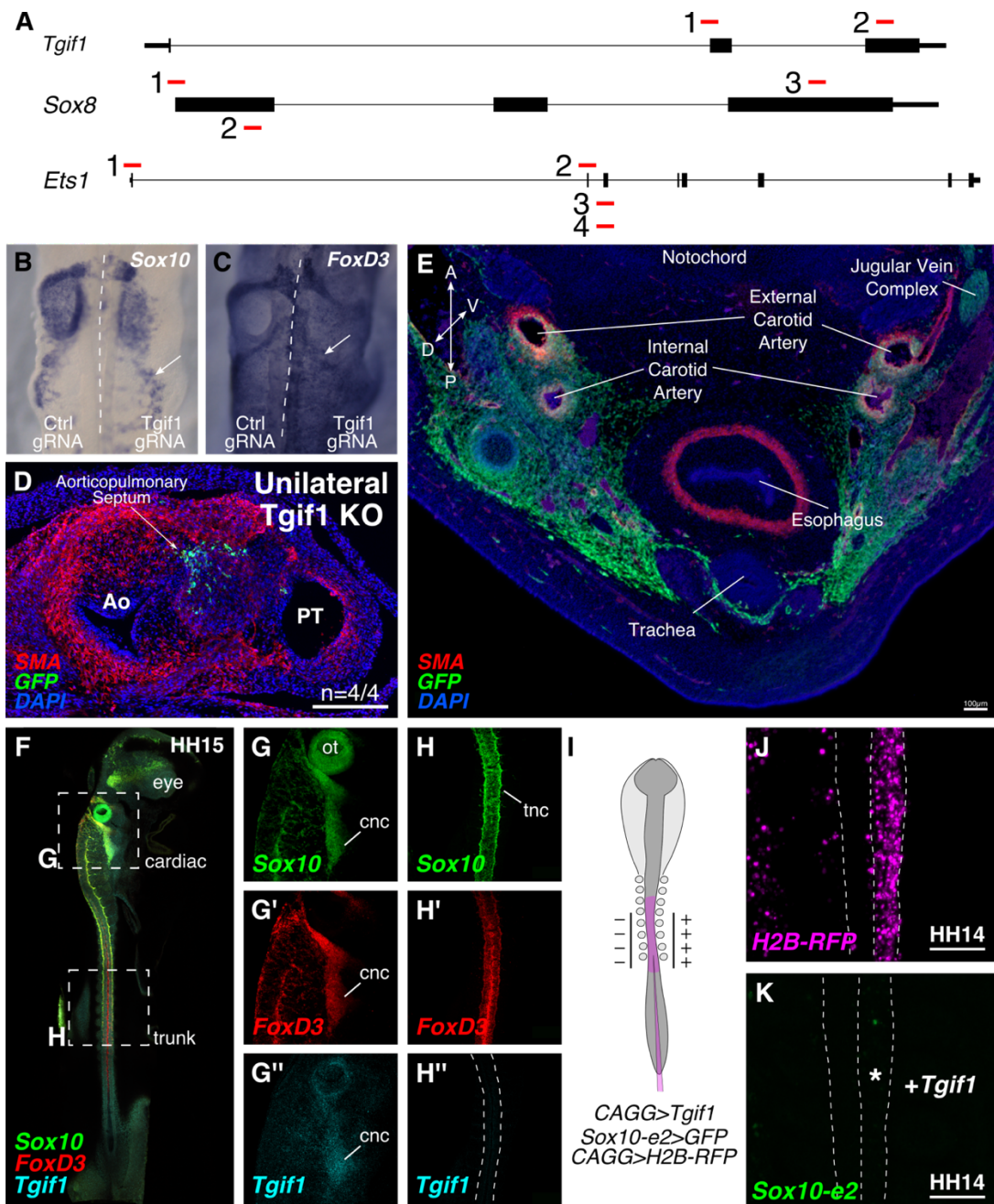
Shashank Gandhi, Max Ezin, and Marianne E. Bronner

Supplementary figure titles and legends



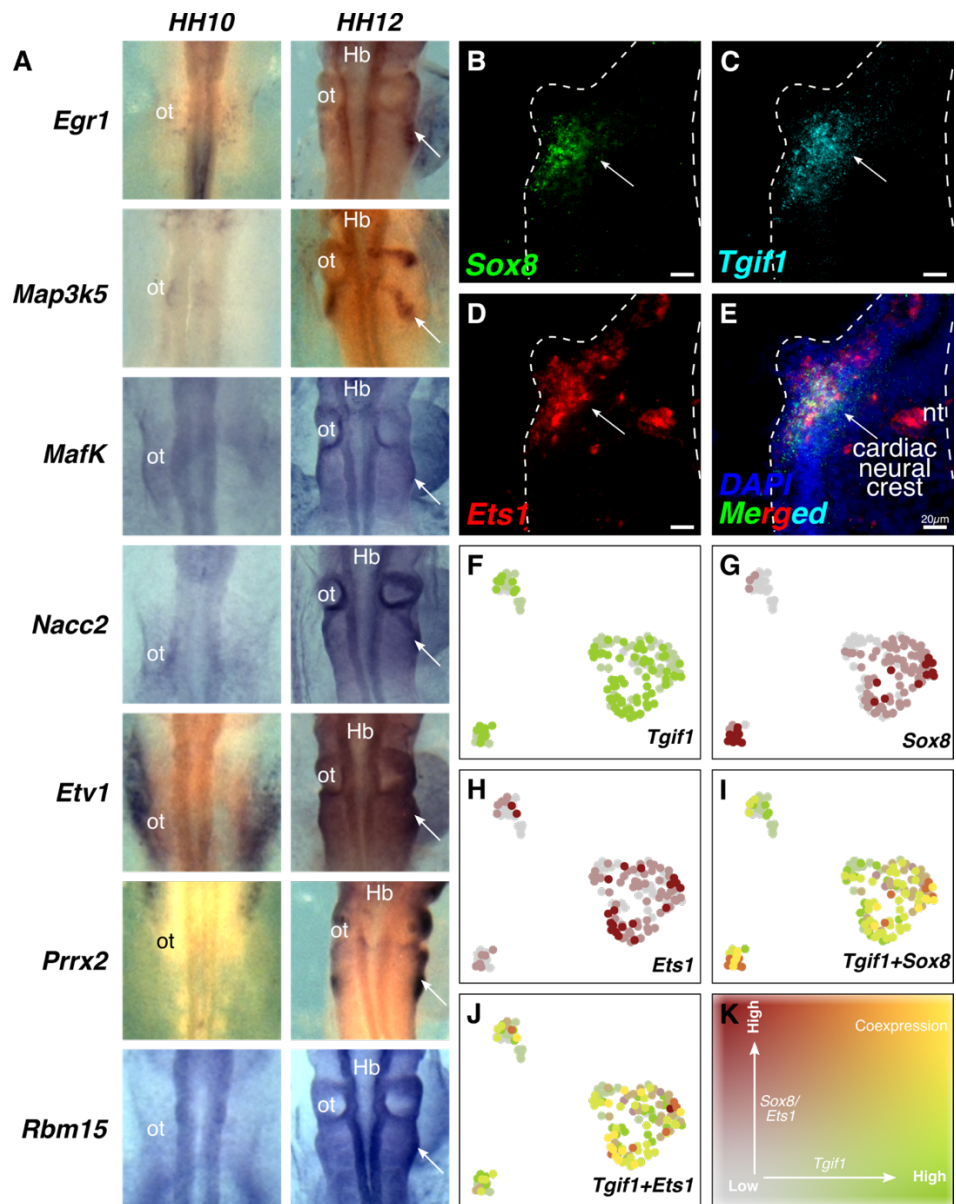
Supplementary Figure S1 (related to figure 1, 2): Effect of ablations on cardiovascular development, and quality control on bulk and single cell RNA-seq data. (A) (left to right) Primary heart tubes of E3 embryos where the cardiac neural folds were left intact, unilaterally ablated on the left, unilaterally ablated on the right, and bilaterally ablated. No difference was observed on the effect of left and right unilateral ablation on primary heart tube looping and elongation. (B) Transverse cross-section through an E6 outflow tract of an embryo where the cardiac neural crest was unilaterally ablated on the left side exhibits Double Outlet Right Ventricle (DORV). (C-D) Hierarchical clustering (C) and Principal Component Analysis (D) done on the two cardiac and three trunk neural crest libraries prepared for population-level transcriptional profiling shows

high correlation between replicates. (E-G) Quality control on SMART-seq sequencing data showing the number of genes identified per cell (E, G) and high correlation between mapped and sequencing reads (F). (H) Scatter plot comparing genes enriched in the bulk and single cell RNA-seq datasets. (I) Genes validated in main figure 2 using in situ hybridization were highly expressed across C1, C2, and C4, with lower expression levels in the ectomesenchymal C3.



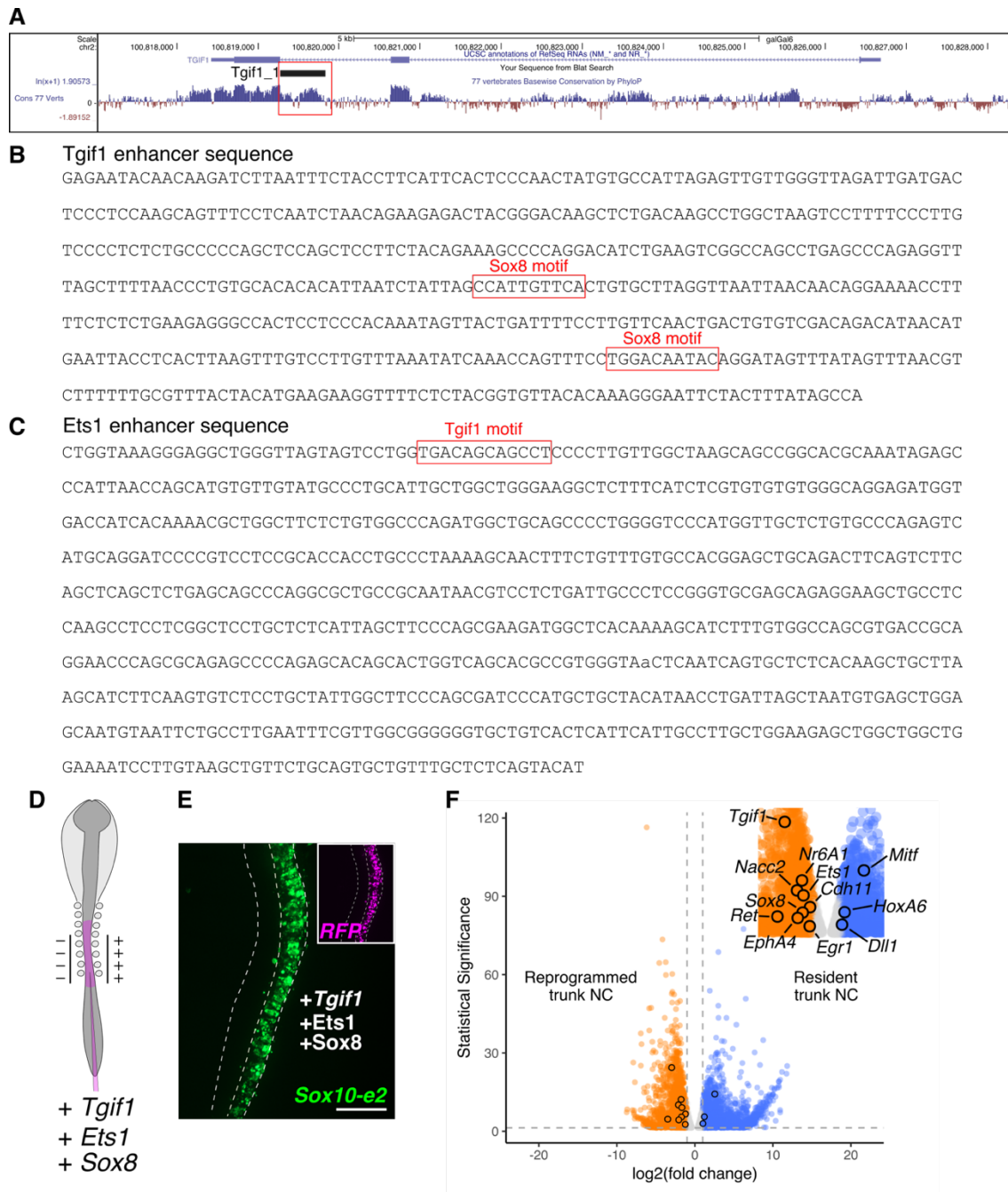
Supplementary Figure S2 (related to figure 3, 4): Role of *Tgif1* in cardiac neural crest. (A) Loci for *Tgif1*, *Sox8*, and *Ets1* with gRNA target sites highlighted in red. 2 gRNAs for *Tgif1*, 3 gRNAs for *Sox8*, and 4 gRNAs for *Ets1* were designed. (B-C) Loss of *Tgif1* resulted in reduced expression of pan-neural crest markers *Sox10* (B) and *FoxD3* (C), but little difference was observed in the number of cells. (D) The outflow tract of

embryos that were unilaterally grafted with a *Tgif1* knockout dorsal cardiac neural fold septated properly. (E) Cross-section through the neck of a chimera where *Tgif1* knockout dorsal cardiac neural folds were bilaterally grafted in the embryo. Cardiac neural crest cells surrounded in the internal and external carotid artery, but failed to form the outflow tract septum. (F-H) Hybridization Chain Reaction in a HH15 embryo shows expression of *Sox10* (G,H) and *FoxD3* (G',H') in both cardiac and trunk neural crest cells. *Tgif1* was expressed in the cardiac region (G''), but absent from the trunk neural crest (H''). (I-K) Electroporation strategy (I) to check the effect of ectopic expression of *Tgif1* on resident trunk neural crest cells. DNA solution containing expression constructs for *Tgif1*, *Sox10-e2>GFP*, and H2B-RFP were injected in the lumen of the neural tube of an HH9+ embryo. The electroporation efficiency was checked using H2B-RFP expression (J). Overexpression of *Tgif1* failed to turn on the *Sox10-e2* enhancer in the trunk neural crest (K).



Supplementary Figure S3 (related to figure 5): Identification of cardiac neural crest subcircuit genes. (A) *in situ* hybridization against genes enriched in the cardiac compared to trunk neural crest that are expressed in migratory (HH12) but not premigratory (HH10) cardiac neural crest. (B-E) Transverse cross-sections through a HH12 embryo where *Tgif1*, *Sox8*, and *Ets1* transcripts were labeled using Hybridization Chain Reaction. Cardiac neural crest cells from the post-otic r6 stream showed

overlapping expression of the three genes. (F-K) Coexpression analysis of *Tgif1* with *Sox8* and *Ets1* using the SMART-seq data. Both genes were abundant in a high proportion of cardiac neural crest cells profiled using single cell RNA-seq.



Supplementary Figure S4: Investigation of functional relationships between cardiac neural crest subcircuit genes and identification of downstream targets (related to figure 5). (A) UCSC genome browser track shows an evolutionary conserved region in the second intron of *Tgif1*. (B-C) The *Tgif1* (B) and *Ets1* (C) enhancer sequence that recapitulated expression of the respective genes in the cardiac neural crest at HH12.

Sox8 binding sites in the Tgif1 enhancer and Tgif1 binding site in the Ets1 enhancer are labeled. (D) Electroporation strategy for isolating reprogrammed trunk neural crest cells for transcriptional profiling. (E) Dorsal view of trunk neural crest from a representative HH14 embryo transfected with expression constructs for Sox8, Tgif1, and Ets1 together with Sox10-e2 enhancer-mediated GFP expression (Scale bar = 100 μ m). (F) Volcano plot showing genes differentially expressed in resident trunk (blue) and reprogrammed trunk (orange) neural crest cells. Inset – genes included in the heatmap in figure 5T are labeled.